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Characterization and comparison of hydrogels anchored with a tridentate, tetradentate and pentadentate chelators

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Recent developments of new sorbents and metal chelation have increased the popularity of Immobilized Metal-Affinity Chromatography (IMAC). Investigation of alternative matrices such as hydrogels has led to identification of techniques that has the potential to overcome many of the disadvantages associated with packed bed chromatography such as low protein loading, elution conditions, regeneration and cost. The advantages support the use of these systems for development of large-scale purification application for industrial usage[1]. Since numerous neighboring histidine residues are uncommon among naturally occurring proteins for selectivity and isolation efficiency, it is important to develop systems that are efficient when there is a low number of histidine residues. Membrane chromatography is especially suited for large-scale processes and is based on the use of thin layers of finely organized and well-controlled macroporous polymeric stationary phases [1]. Due to the macroporous structure of the membrane support, membrane chromatography has lower pressure drops, higher flow rates, and higher productivity than column chromatography. The inclusion of immobilized metal affinity groups as fixed carriers in hyrodgel membrane matrices bound with a divalent metal enhances the separation characteristics of the membrane. Hydrogels are attractive materials as a chassis for the IMAC system due to its simple processability, biocompatibility and minor reactivity to environmental change (e.g. temperature, pH, etc.) [2].

In this study, Polyvinyl alcohol (PVA) was used as the hydrogel membrane and was subsequently functionalized with a spacing element (1,4-butanediol diglycidal ether (1,4-BDE)), chelating ligand (iminodiacetic acid (IDA), nitrilotriacetic acid (NTA) or N-(2-hydroxyethyl) ethylenediamine N-N'-N' triacetic acid (HEDT)) and a divalent metal (Cu^{2+} or Ni^{2+}) (Figure 1). The objective of this study is to characterize and compare the transport properties of immobilized metal affinity hydrogel membranes that are anchored using a tridentate, tetradentate and pentadentate chelator. Atomic Absorption Spectroscopy (AAS) and Fourier Transform Infrared spectroscopy (FTIR) and Equilibrium Solution Content (ESC) were used to characterize and determine functionalization of the hydrogels.

AAS was used to determine the bound and unbound metal concentration and to make initial determinations regarding the degree of functionalization of the membrane. Metal concentrations examined were: .01M, .05M, .1M, and .5M. Bound Ni^{2+} (BNC) and Cu^{2+} (BCC) content for the functionalized membranes are not shown. Metal analysis through AAS demonstrate that affinity membranes using either chelator can efficiently bind Cu^{2+} or Ni^{2+} at least 4mg of metal per gram of membrane from an aqueous solution. The results demonstrated that 0.05M metal (II) solution was sufficient for affinity chromatography for all three chelators.

Preliminary results from FTIR spectra show all major peaks (3300, 2940, 1731, 1141 and 1087) related to hydroxyl and acetate groups which is indicative of a PVA hydrogel crosslinked with glutaraldehyde (Figure 2) [3]. These results proved that cross-linking had occurred with the change in chemical structure of the hydrogel however, further analysis is needed to confirm the functionalized affinity membrane. The data also implied that few differences occur in the hydrogels' structure when the chelators are attached.

Hydrogels are a three-dimensional network of crosslinked polymers with an open mesh system that are hydrophilic; there water-absorbing capacity is between 20–100% of their total weight. The equilibrium swelling of hydrogels in water and different buffers with varying pH values (5, 6.5, and 8) indicated that there was sustained expansion of the films in different pH solutions (Data not shown). The control and affinity membrane in equilibrium solution content are similar

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for different pH buffers. As expected, swelling is greater in water than in buffer for all membranes due to ions replacing water molecules.

This study provides evidence that a functionalized membrane using different chelators was designed and successfully synthesized. Thus, results suggest that the metal-chelate complex is sufficient for bioseparations. Chicken egg white lysozyme (CEWL) was the solute used as the model protein to characterize the solubility in control (membranes without metal attached) and affinity membranes (membranes with metal attached). An adsorption study using chicken egg white lysozyme (CEWL) was conducted to examine the effects of pH on solubility in the control and affinity membranes of the PVA-BDE-IDA-Cu (Figure 3). Results have demonstrated that Cu^{2+} affinity membranes increase the solubility of CEWL. Also, PVA-BDE-NTA membrane was used for protein separation. However, the Cu² affinity membranes attached more protein showing while the Ni² affinity membranes resembled control membranes. Conclusion can be drawn that buffer conditions interfered with protein attachment (Figure 4).

Atomic Absorption spectroscopy confirmed that a Ni and Cu affinity hydrogels were successfully created. As shown before, NTA proved to be very effective in chelating Ni^{2+} and the data suggests it has a preference for Ni^{2+} over Cu^{2+}. Future studies will include determination of binding constants (K_d) of NTA for Ni^{2+} and Cu^{2+}. Increased amount of NTA did not increase the amount of bound Ni^{2+} (data not shown). Additionally, it is concluded that data from adsorption and metal analysis results indicate Me^{2+} leaching is occurring during protein adsorption experiments of NTA-Ni membranes. This may be due to the high concentration of NaCl (0.5M). There is a strong interaction between the Me^{2+} and protein, but the experimental conditions may be affecting the spacer element, BDE. Buffer conditions will be investigated to prevent such occurrence. Further evidence will be pursued to elucidate the environmental conditions necessary for protein attachment to the NTA-Ni membrane. It has been reported that salt (NaCl) content may cause adverse effects during protein separation [4].

Future studies will be performed using .05M Ni^{2+} to complete sorption studies using the model protein chicken egg white lysozyme (CEWL) and *Streptococcus pnuemoniae* pneumolysin with varying conditions. *Streptococcus pnuemoniae* pneumolysin plays a critical role in the virulence of an eye infection caused by this gram-positive bacterium. A histidine-tagged pneumolysin has been constructed, and the affinity hydrogels will be optimized for protein purification from expression in *E. coli*. The pure protein will be added directly to the eye to examine its toxicity properties.

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